TranSignal™
TF Protein Array

Cat. # MA3501 - MA3508
Product User Manual
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Transcending boundaries ...

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1. INTRODUCTION

The human genome comprises an estimated 30,000 genes, 2,000 of which encode proteins involved in the control of gene expression. These proteins, known as transcription factors (TFs), are responsible for the who, what, when, where, and how of gene expression: which genes are expressed in what cell types, under what environmental conditions, and at what stages of development. The activity of TFs is highly regulated by various mechanisms, including protein induction, modification, translocation, degradation, and inhibition (1).

Before activated TFs can impose regulation on the basal transcriptional machinery, they either bind to their corresponding specific *cis*-elements, or interact with other TF proteins. This interaction usually requires cofactors to bridge the TF proteins and the components of the basal transcriptional machinery. The interactions between TFs, cofactors, and basal transcriptional machinery create a complex, multidimensional network (2).

Identifying and characterizing the interaction network in the context of different cellular environments will allow us to understand the network structure, as well as the cellular signal-induced changes in this structure. Tools for studying protein-protein interactions—especially interactions involving TF proteins—are essential for understanding gene expression and regulation.

Novel method for investigating protein-protein interactions

Traditionally, protein-protein interactions have been studied by pull-down assay, coimunoprecipitation, super-gel shift, and the yeast two-hybrid system. But because these methods are notoriously time-consuming and inefficient, they are not conducive to mapping the intricate network of protein-protein interaction. That's where the TranSignal™ TF Protein Array comes in. This method enables you to determine how a particular protein interacts with multiple other proteins—in a single detection experiment.

Figure 1 illustrates how this simple procedure works. The array membrane is spotted with transcription factor proteins, which are expressed from full length TF cDNAs, with an N-terminal His Tag. A protein of interest is

used as bait to search for interactions with the immobilized proteins. Interactions can be assessed either by using an antibody to the protein of interest, or with antibody to protein tags such as HA, GST or biotin. The signal is visualized via chemiluminescent detection.

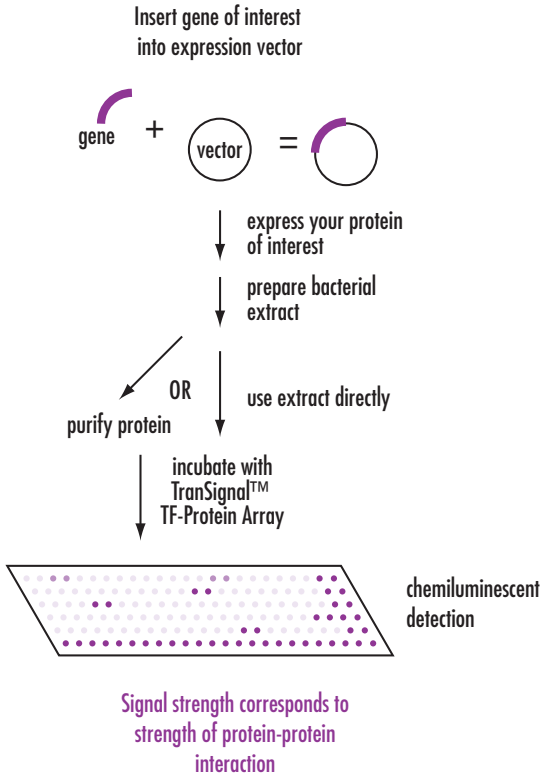


Figure 1: Flow chart of the TranSignal™ TF Protein Array protein interaction

Novel method to characterize gene promoter regions

The TranSignal™ TF Protein Array provides a method by which you can very quickly survey direct interactions between TF proteins and their complimentory cis-binding element. This means that by simply producing a biotinylated version of your promoter region of interest you can asses which transcription factors arrayed on the membrane have the potential to bind to this region. Once these have been identified it starts to give clues about which transcription factors, and their associated signaling pathways, might be regulating the expression of your gene of interest.

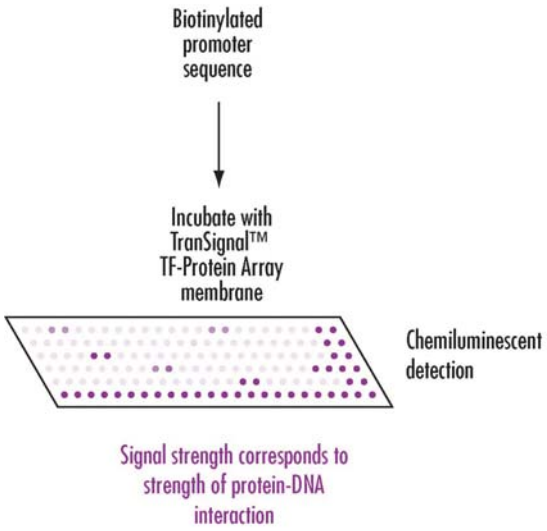


Figure 2: Flow chart of the TranSignal™ TF Protein Array DNA Promoter interaction

2. CHOOSING AN ANTIBODY DETECTION METHOD

IMPORTANT: Read this section before beginning your experiment!
To detect interactions between your protein of interest and the TF proteins immobilized on the membrane, you will need to use either an antibody against your protein of interest or a tag antibody, such as HA, biotin or GST. As a negative control, incubate the TranSignal TF Protein Array membrane with antibody only (see Appendix B).

2.1 Using an antibody against your protein of interest

If you already have access to your purified protein of interest or bacterial lysate containing the protein of interest, you can get started right away. Simply incubate the array membrane with your purified protein, then detect using the antibody.

2.2 Using a protein tag/antibody combination

If you don't have an antibody against your protein of interest, you will need to use protein with a specific tag, such as HA or GST or biotinylate your protein. **Do not use His tagged proteins for hybridization with the membranes as the proteins on the array are His tagged**. You can then detect hybridized proteins using an antibody against the tag.

2.3 Using a protein tag/antibody combination

We provide a suitable streptavidin - HRP conjugate for use with this kit

3. MATERIALS PROVIDED

For Catalogue items MA3501, MA3502, MA3503 and MA3504 Protein - Protein interactions.

STORAGE CONDITONS: Upon receipt, store array membranes, and all reagents at 4°C. Keep at 4°C until use.

- **TranSignal TF Protein Array** (2 each)
- **1X Blocking Buffer I** (30 ml)
- **1X Blocking Buffer II** (30 ml)
- **20X Wash Buffer** (20 ml)–dilute to 1X with dH_20

- **Detection Buffer A** (600 µl)
- **Detection Buffer B** (600 µl)

For Catalogue items MA3505, MA3506, MA3507 and MA3508 DNA - Protein interactions.

STORAGE CONDITONS: Upon receipt, remove the 5X Blocking Buffer and store at -20°C. Store array membranes, and all other reagents at 4°C.

- **TranSignal TF Protein Array** (2 each)
- **4 well plate** (1 each)
- **5X Binding Buffer** (5 ml)
- **20X Wash Buffer** (20 ml)–dilute to 1X with dH_20
- **Streptavidin - HRP** (20 µl)
- **Detection Buffer A** (600 µl)
- **Detection Buffer B** (600 µl)

Sufficient quantities of each buffer are provided for two assays.

4. ADDITIONAL MATERIALS REQUIRED

4.1 Reagents and Solutions

- **Appropriate primary detection antibody**
- **Anti-rabbit, mouse or goat - HRP conjugate**

4.2 Materials and Equipment

- **Microcentrifuge**
- **Orbital shaker**
- **Plastic Sheets** (e.g., overhead transparencies)
- **Hyperfilm™ ECL** (Amersham, Cat.# RPN1674K) or equivalent

OR

- **Chemiluminescence imaging system** (e.g., FluorChem™ from Alpha Innotech Corp.)

5. INCUBATION OF PROTEIN WITH THE ARRAY MEMBRANE

Use this protocol with catalogue items MA3501, MA3502, MA3503 and MA3504.

This section describes incubating bacterial extract containing your protein of interest or purified protein with the array membrane. Note that the array membranes are notched at the top right-hand corner for orientation purposes.

Note: *Be sure that the membrane is fully submerged in assay buffer at all times. Never let the membrane dry out.*

5.1 Place each membrane into a container containing 4 ml of 1X Blocking Buffer I.

5.2 Place the tray on a shaker and incubate for 2 hr at room temperature.

5.3 Dilute the bacterial extract, containing overexpressed protein to a final concentration of 60 µg/ml in 4 ml of 1X Blocking Buffer I. If you have purified protein, dilute 3 -5 µg purified protein in 4 ml of 1X Blocking Buffer I.

5.5 Incubate the membrane with the diluted bacterial extract or purified protein at room temperature for 2 hr with gentle shaking.

5.6 Wash the membrane twice with 4 ml of 1X Wash Buffer for 5 min (each wash) at room temperature.

5.7 Incubate the membrane with 4 ml of 1X Blocking Buffer II containing primary antibody:

Note: Use an amount of the antibody suitable for western blot detection of the protein or protein tag, specified by the manufacturer.

5.8 Incubate the membrane with the primary antibody solution for 2 hours at room temperature,

5.9 Wash the membrane twice with 4ml 1X Wash Buffer for 5-min (each wash).

5.10 Incubate the membrane with the appropriate secondary antibody diluted 1:5,000–1:15,000 in 4ml 1X Blocking Buffer II for 1 hr at room temperature.

5.11 Wash the membrane four times with 4 ml of 1X Wash Buffer for 5 min (each wash) at room temperature.

6. DETECTION

Important: Do not let the membrane dry out during detection.

6 .1 Prepare 500µl of detection solution by combining 250µl Detection Buffer A and 250 µl of Detection Buffer B per membrane.

6.2 Using forceps to hold the notched corner, carefully remove each membrane from its tray. Drain the excess Wash Buffer from the membrane by touching the edge against tissue. Place membrane protein-side-up on a clean plastic sheet protector or overhead transparency, by orienting the notch to the top, right-hand corner.

6.3 Pipet the mixed Detection Buffers onto the membrane. Overlay the membrane with a second plastic sheet and ensure that the buffer mixture is evenly distributed over the membrane without air bubbles.

6.4 Incubate for 5 min at room temperature.

6.5 Remove excess substrate by gently applying pressure to the top sheet. Using a paper towel, remove excess detection solution remaining on the surface of the sheets.

6.6 Expose the membranes using either Hyperfilm™ ECL or a chemiluminescence imaging system, such as the FluorChem™ imager from Alpha Innotech Corp. In either case, we recommend that you try several different exposures of varying lengths of time (e.g., 30 sec–5 min).

7. INCUBATION OF DNA WITH THE ARRAY MEMBRANE

Follow this protocol for use with catalogue items, MA3505, MA3506, MA3507 and MA3508

This section describes incubating a biotinylated oligo of interest with the array membrane. We recommend that you use a 5'biotinylated Oligo for this assay.

Note: *Be sure that the membrane is fully submerged in assay buffer at all times. Never let the membrane dry out.*

7.1 Place each membrane into a container containing 4 ml of 1X Binding Buffer.

7.2 Place the tray on a shaker and incubate for 2 hr at room temperature.

7.3 Dilute 50 - 200 ng of biotinylated DNA probe in 4 ml of 1X Binding Buffer. For the competition assay cold probes should be in at least 5 X excess of the biotinylated probe (0.25- 1 µg)

7.5 Incubate the membrane with the diluted DNA probe at room temperature for 30 min with gentle shaking.

7.6 Wash the membrane two times with 4 ml of 1X Wash Buffer for 3 min (each wash) at room temperature.

7.7 Dilute the Streptavidin - HRP (1:1000 fold) in 1X Wash Buffer and incubate the membraneg with the diluted Streptavidin - HRP for 30 min at roomtemperature.

7.8 Wash three times with 4 ml of 1X Wash Buffer for 5 min (each wash) at room temperature.

7.9 Continue with step 6.1 for detection.

8. TROUBLESHOOTING GUIDE

Problem	Cause	Recommendation
Weak or no signal	Not enough protein.	Check protein concentration by running the sample on SDS-PAGE.
		Check construct by DNA sequencing. Make sure the DNA insert is in the right frame and that the protein expresses properly.
	Tag is partially hidden.	Protein binding may be hindered by a partially hidden tag. Try using a higher concentration (5-10X of the bacterial lysate) or longer binding time.
High background	Concentration of bacterial lysate is too high.	Further dilute bacterial lysate or use purified protein.
	Antibody concentration is too high.	Further dilute the antibody.
		Dilute the Detection Buffer.
Uneven background	Membrane dried out during incubation.	Keep the membrane fully submerged in solution during all incubation steps.
	Volume of blocking solution, bacterial lysate or antibody is too low.	Increase the volume to make sure that the membrane is fully submerged during incubation.
	Volume of detection buffer is too low.	Increase the volume to make sure that the membrane surface is fully covered.
	Air bubbles on membrane surface during detection.	Remove air bubbles from membrane surface.

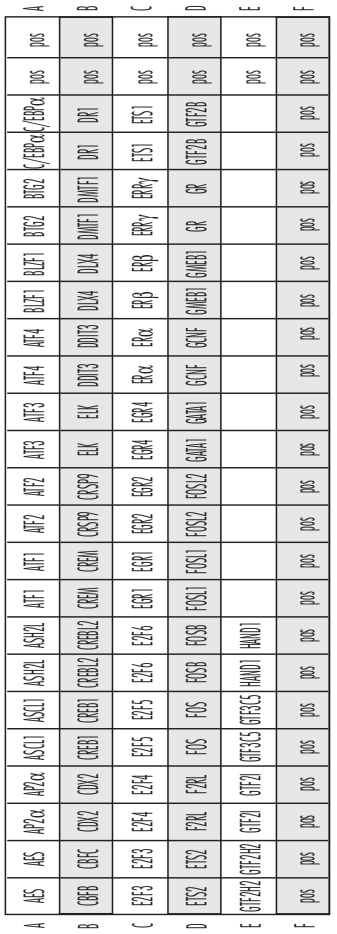
9. REFERENCES

1. Sawyer TK (2001) Decifering therapeutic targets. *BioTechniques* 30: 1086-1090.

2. Chan, H M., and La Thangue, N. B. (2001) p300/CBP protiens: HAT for trancriptional bridges and scaffolds. *J Cell Science* 114: 2363-2373.

3. Chunhong Yan, Heng Wang, and Douglas D. Boyd (2002) ATF3 Represses 72-kDa Type IV Collagenase (MMP-2) Expression by Antagonizing p53-dependent *trans*-Activation of the Collagenase Promoter. *J Bio Chem* 277: 10804-10812.

APPENDIX A: Schematic diagram of the TranSignal TF Protein Array Version I

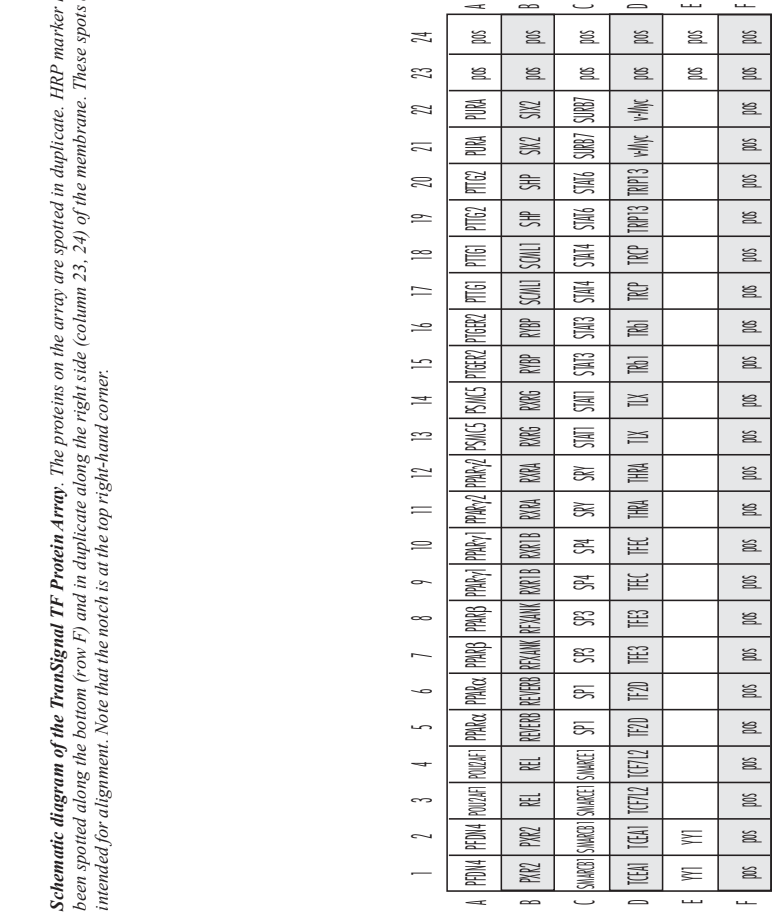


Schematic diagram of the TranSignal TF Protein Array*: The proteins on the array are spotted in duplicate. HRP marker has been spotted along the bottom (row F) and in duplicate along the right side (column 23, 24) of the membrane. These spots are intended for alignment. Note that the notch is at the top right-hand corner.*

APPENDIX B: Schematic diagram of the TranSignal TF Protein Array Version II

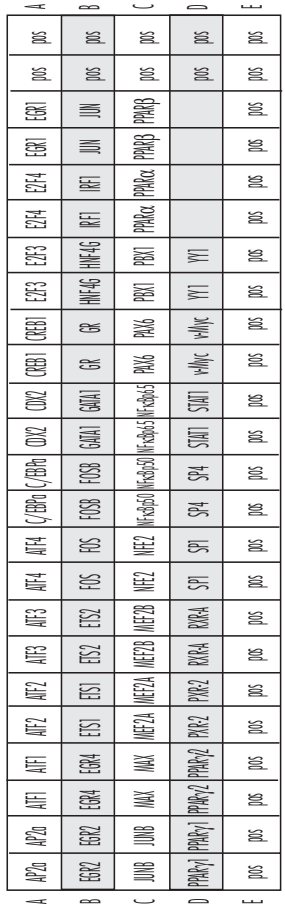
Schematic diagram of the TranSignal TF Protein Array*. The proteins on the array are spotted in duplicate. HRP marker has been spotted along the bottom (row F) and in duplicate along the right side (column 23, 24) of the membrane. These spots are intended for alignment. Note that the notch is at the top right-hand corner.*

APPENDIX C: Schematic diagram of the TranSignal TF Protein Array Version III



Schematic diagram of the TranSignal TF Protein Array*. The proteins on the array are spotted in duplicate. HRP marker has been spotted along the bottom (row F) and in duplicate along the right side (column 23, 24) of the membrane. These spots are intended for alignment. Note that the notch is at the top right-hand corner.*

APPENDIX D: Schematic diagram of the TranSignal TF Protein Array Version IV

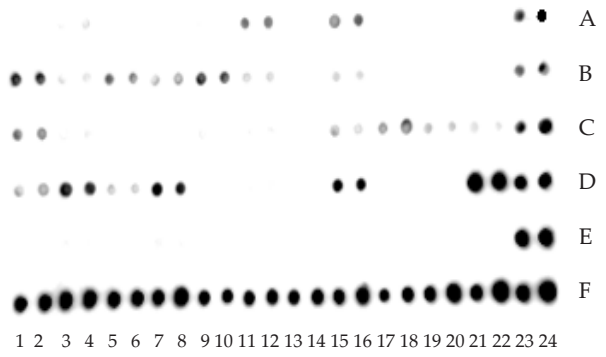


	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	AP2α	AP2α	ATF1	ATF1	ATF2	ATF2	ATF3	ATF3	ATF4	ATF4	C/EBPα	C/EBPα	COX2	COX2	CREB1	CREB1	E2F3	E2F3	E2F4	E2F4	EGR1	EGR1	pos	pos
B	EGR2	EGR2	EGR4	EGR4	ETS1	ETS1	ETS2	ETS2	FOS	FOS	FOSB	FOSB	GATA1	GATA1	GR	GR	HNF4G	HNF4G	IRF1	IRF1	JUN	JUN	JUN	JUN
C	JUNB	JUNB	MAX	MAX	MEF2A	MEF2A	MEF2D	MEF2D	NFE2	NFE2	NFκBp50	NFκBp50	NFκBp65	NFκBp65	PAX6	PAX6	PBX1	PBX1	PPARα	PPARα	PPARβ	PPARβ	PPARδ	PPARδ
D	PPARγ1	PPARγ1	PPARγ2	PPARγ2	PXR2	PXR2	RXRA	RXRA	SP1	SP1	SP4	SP4	STAT1	STAT1	vHNF1	vHNF1	YY1	YY1	pos	pos	pos	pos	pos	pos
E	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos

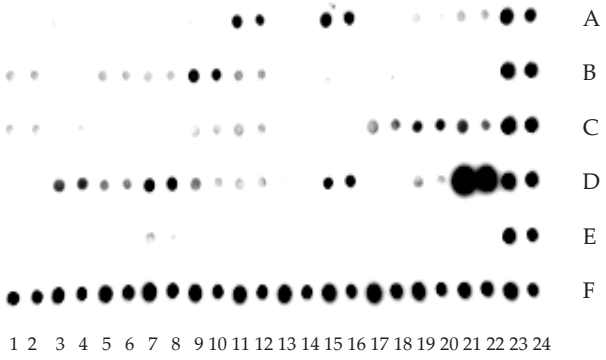
Schematic diagram of the TranSignal TF Protein Array. *The proteins on the array are spotted in duplicate. HRP marker has been spotted along the bottom (row E) and in duplicate along the right side (column 23, 24) of the membrane. These spots are intended for alignment. Note that the notch is at the top right-hand corner.*

APPENDIX E: Typical Results of the TranSignal TF Protein Array when screening protein - protein interactions

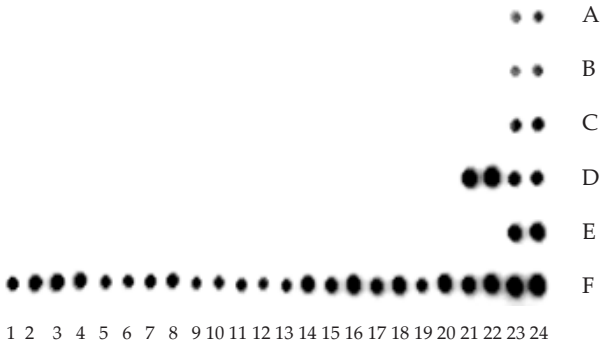
A. Purified p53 Protein



B. Bacterial Lysate containing p53

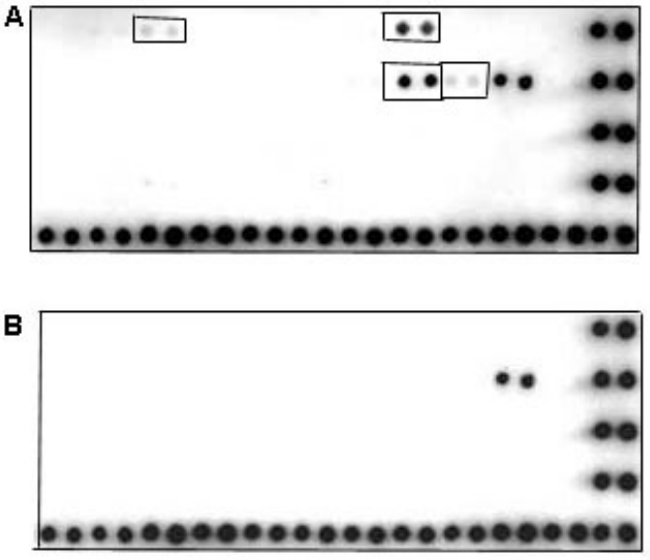


C. Negative Control



Typical results obtained with the TranSignal TF Protein Array. *p53-expressing bacterial lysate (A), p53 purified protein (B), and buffer only (C) were incubated with the TranSignal TF Protein Array membrane for 2 hours and detected by p53 monoclonal antibody (Upstate Biotechnology) and anti-mouse Ig HRP conjugate (Amersham). Images were acquired using FluorChem™ imager (Alpha Innotech). As reported in the literature, ATF3 (position A11, A12) interacts with p53 (3).*

APPENDIX F: Typical Results of the TranSignal TF Protein Array when screening protein - DNA interactions



Typical results obtained with the TranSignal TF Protein Array IV. *The TF protein array IV was incubated with the biotinylated PCR product of the rat PEPCK Promoter Region (-1 to -465) alone (Figure A) and both biotinylated and unlabeled PEPCK promoter (Figure B.). Images were acquired using FluorChem™ imager (Alpha Innotech). Specific Protein/DNA interactions (boxes) were competed out with unlabeled probe, whereas non-specific binding of the probe was not.*

NOTES: